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(54) Title: THE USE OF DES-ASPARTATE-ANGIOTENSIN I AS AN ANTI-CARDIAC HYPERTROPHIC AGENT

(57) Abstract

The use of des-Aspartate-angiotensin I (Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu) as an anti-cardiac hypertrophic agent is described. The compound, given either intravenously or orally, prevented the development of experimentally-induced cardiac hypertrophy in rats. Its action was dose-dependent and the maximum anti-cardiac hypertrophic effect was obtained at a dose of (i) 180 mg/day when given intravenously, and (ii) 285 mg/day when given orally.

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THE USE OF DES-ASPARTATE-ANGIOTENSIN I AS AN ANTI-CARDIAC HYPERTROPHIC AGENT

TECHNICAL FIELD

This invention relates to an anti-cardiac hypertrophic agent.

BACKGROUND ART

The interest in des-Aspartate-angiotensin I, a nine amino acid angiotensin peptide (Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu), as a peptide of the renin-angiotensin system was first generated when Blair-West and colleagues (Blair-West et al, *J. Clin. Endocrinol. Metab.*, 32:575-578 (1971)) postulated a biosynthetic pathway for the production of angiotensin III by enzymatic NH_2-terminal degradation of angiotensin I to the nonapeptide and sequential action of angiotensin converting enzyme on this nonapeptide to produce the heptapeptide. Since then des-Aspartate-angiotensin I has been shown to be an excellent substrate of plasma and pulmonary angiotensin converting enzyme (Tsai et al, *J. Med. Chem.*, 18:1180-1183 (1975)) and that its pressor and steroidogenic actions are dependent on its conversion to angiotensin III (Campbell et al, *Endocrinology*, 100:46-50 (1977)). Recently, we found that homogenates of rat aorta and hypothalamus degrade exogenous angiotensin I to mainly des-Aspartate-angiotensin I instead of angiotensin II and the enzyme responsible for the degradation was a specific aminopeptidase that was not inhibited by amastatin, bestatin and EDTA (Sim, *Biochem. Pharmacol.*, 45:1524-1527 (1993); Sim et al, *Blood Pressure*, 3:260-264 (1994) and Sim et al, *Biochem. Pharmacol.*, 48:1043-1046 (1994)). Des-Aspartate-angiotensin I has also been shown to attenuate the pressor action of angiotensin II and angiotensin III in the brain (Sim and Radhakrishnan, *Eur. J. Pharmacol.*, 257:R1-R3 (1994)). Peripherally, it is able to potentiate the contractile action of angiotensin II on the rabbit aortic ring but to attenuate the contractile action of angiotensin III in the same tissue (Sim and Yuan, *Eur. J. Pharmacol.*, 287:175-178 (1995)). These recent findings of ours seem to indicate that des-Aspartate I is a functional peptide that may have undefined specific actions in ensuring the normal functioning of the cardiovascular system.

DISCLOSURE OF INVENTION

In the course of studying the cardiovascular pharmacology of des-Aspartate-angiotensin I, the nonapeptide has been found to attenuate significantly the experimentally-induced cardiac hypertrophy in rat. It has been surprisingly discovered that des-Aspartate-angiotensin I is effective in accordance with the present invention at an exceeding low dose, i.e. an i.v. dose of 180 ng (given over a period of 4 hours) per day for four days attenuates significantly the experimentally-induced cardiac hypertrophy in rats. Another significant finding is that, despite being a peptide, des-Aspartate-angiotensin I is equally effective in attenuating the cardiac hypertrophy when given orally at 285 mg per day for four days. These findings show that des-Aspartate-angiotensin I is a highly specific anti-cardiac hypertrophic agent and is effective at concentrations that produce minimum or no secondary effects.

Therefore, the present invention is directed to the use of des-Aspartate-angiotensin I as an anti-cardiac hypertrophic agent or in the preparation of an anti-cardiac agent, for either the prevention or treatment of cardiac hypertrophy or a pharmaceutical composition for preventing or treating cardiac hypertrophy comprising an effective amount of des-Aspartate-angiotensin I and a pharmaceutically acceptable carrier or diluent or a method for preventing or treating cardiac hypertrophy, which comprises administering to a subject in need of treatment an effective amount of des-Aspartate-angiotensin I or a packaged pharmaceutical composition for preventing or treating cardiac hypertrophy comprising a container suitable for storing a pharmaceutical preparation, an effective amount of des-Aspartate-angiotensin I in said container, and instructions associated with said container giving instructions for the use of said des-aspartate-angiotensin I for preventing or treating cardiac hypertrophy.

MODES FOR CARRYING OUT THE INVENTION

In the practice of the method of the present invention, an effective amount of des-Aspartate-angiotensin I or a derivative or salt thereof, or a pharmaceutical composition containing the same, as described below, is administered to a subject, such as a human patient, via any of the usual and acceptable methods known in the art, either singly or in combination with other pharmaceutical agents such as captopril or other angiotensin converting enzyme inhibitors. The compound or composition can thus be administered orally, by suppository, or parenterally (e.g., intramuscularly, intravenously, subcutaneously

or intradermally), and in the form of either solid or liquid dosage including tablets, suspensions, or solutions, as is discussed in more detail below. The administration can be conducted in single dosage form with continuous therapy or in single dose therapy ad libitum.

Useful pharmaceutical carriers for the preparation of the pharmaceutical compositions hereof can be solids, liquids or mixtures thereof; thus, the compositions can take the form of tablets, pills, capsules, powders, enterically coated or other protected formulations, sustained release formulations, erodible formulations, implantable devices or components thereof, microsphere formulations, solutions, suspensions, elixirs, aerosols, and the like.

Water, saline, aqueous dextrose, and glycols are preferred liquid carriers, particularly (when isotonic) for injectable solutions. The carrier can be selected from various oils including those of petroleum, animal, vegetable or synthetic origin, for example, peanut oil, soybean oil, mineral oil, sesame oil, and the like. Suitable pharmaceutical excipients include starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol, and the like. The compositions may be subjected to conventional pharmaceutical expedients such as sterilization and may contain conventional pharmaceutical additives such as preservatives, stabilizing agents, wetting or emulsifying agents, salts for adjusting osmotic pressure, buffers, and the like. Suitable pharmaceutical carriers and their formulations are described in Martin, "Remington's Pharmaceutical Sciences", 15th Ed.; Mack Publishing Co., Easton (1975); see, e.g., pp. 1405-1412 and pp. 1461-1487. Such compositions will, in general, contain an effective amount of the active compound together with a suitable amount of carrier so as to prepare the proper dosage form for proper administration to the host.

In one preferred embodiment, the therapeutic methods of the present invention are practiced when the relief of symptoms is specifically required or perhaps imminent; in another preferred embodiment, the method hereof is effectively practiced as continuous or prophylactic treatment.

In the practice of the therapeutic methods of the invention, the particular dosage of pharmaceutical composition to be administered to the subject will depend on a variety of

considerations including the stage of the disease or condition, the severity thereof, the schedule of administration, the age and physical characteristics of the subject, and so forth. Proper dosages may be established using clinical approaches familiar to the medicinal arts.

Although the initial work was conducted in a rat experimental model, it is expected that the invention can be utilized in various mammals including, but not limited to, mice, rabbits and humans.

Example

Sources of Materials: Des-Aspartate-angiotensin I was obtained from Bachem (Bubendorf, Switzerland). Des-Aspartate-angiotensin I can be prepared by techniques well known in the art. Adult Sprague Dawley rats (250-300 g) were obtained from the Animal Centre, National University of Singapore.

Induction of Cardiac Hypertrophy: The abdominal aorta of each animal was co-arcted to the size of a 23-gauge hypodermic needle with a silk thread according to the method described by Everett et al (*Hypertension*, 23:587-592 (1994)). Briefly, each animal was anaesthetized with pentobarbital (5 mg/100 g, i.p.). An incision was made in the ventral abdominal wall to access the suprarenal portion of the abdominal aorta. This portion of the abdominal aorta was dissected free and a blunt 23-gauge needle was placed adjacent to the aorta. A ligature was placed around the blunt needle and the aorta. The blunt needle was then removed, leaving the aorta constricted to the size of the needle. The resulting coarctation resisted the normal flow of blood from the heart to the lower portion of the body and placed an extra load on the heart. This extra load is believed to cause hypertrophy of the heart, especially the left ventricle.

Administration of Des-Aspartate-Angiotensin I: Following the operation, each animal was administered one of the various doses of des-Aspartate-angiotensin I (dissolved in saline) per day for four days. The nonapeptide was administered either intravenously via a femoral vein catheter which was implanted during the co-arctation operation or orally via a 1 ml syringe with a blunt needle. The intravenous administration was carried out using a microinjector which delivered 10 ml of the peptide solution per hour for four hours. For oral administration, the peptide was dissolved in 0.5 ml saline. Control animals with co-arcted

abdominal aorta were administered saline instead of the peptide solution. Sham animals were animals that underwent the same surgical operations but their aortae were not co-arcted.

Determination of Cardiac Hypertrophy: On the fourth day following the co-arctation of the abdominal aorta, the animal was again anaesthetized with pentobarbital and the carotid and femoral blood pressure were determined via a carotid artery catheter and a femoral artery catheter, respectively. Each catheter was connected to a Gould Statham (P23 ID) pressure transducer. The transducers were in turn connected to a MacLab Quad Bridge Amplifier coupled to a MacLab/8 Virtual Instrument System which displayed the mean arterial blood pressure in mm Hg. The difference in the two readings indicated the extent of co-arctation. The heart of each animal was then excised and the weight of the ventricles was determined. The index of the ventricle weight (in mg) over the body weight of the animal (in g) was taken as the index of hypertrophy. For sham-operated animals the index was around 2.5, for aorta-co-arcted animal the index was above 3.7.

Results

The results of the study are summarized in Table 1. Des-Aspartate-angiotensin I has been found to be an effective agent in preventing the development of experimentally-induced cardiac hypertrophy. The anti-hypertrophic action is dose-dependent and its maximum action is brought about by an i.v. dose of 180 ng/day for four days or an oral dose of 285 mg/day for four days.

Table 1 Effects of des-Aspartate-angiotensin I on cardiac hypertrophy in rats

Dose		Hypertrophy Index[1]	CBP (mm Hg)	FBP (mm Hg)	BP (mm Hg)
No Administration					
Sham animals		2.53 ± 0.06	129 ± 13	129 ± 13	0
Intravenous Administration					
Control animals		3.77 ± 0.06	151 ± 19	107 ± 22	44
23 ng	(19 pmol)	3.72 ± 0.18	158 ± 17	106 ± 13	52
45 ng	(38 pmol)	3.51 ± 0.09	159 ± 25	119 ± 18	40
90 ng	(76 pmol)	3.47 ± 0.13	156 ± 15	124 ± 14	32
180 ng	(152 pmol)	3.17 ± 0.14	142 ± 22	111 ± 25	31
Oral Administration					
Control animals		3.75 ± 0.06	159 ± 18	110 ± 20	49
64 µg	(63.5 nmol)	3.40 ± 0.10	153 ± 10	89 ± 27	64
128 µg	(125 nmol)	3.23 ± 0.12	163 ± 27	121 ± 30	42
285 µg	(250 nmol)	2.93 ± 0.09	171 ± 24	119 ± 33	52
590 µg	(500 nmol)	3.13 ± 0.22	153 ± 22	110 ± 19	43
1180 µg	(1000 nmol)	3.34 ± 0.16	154 ± 29	107 ± 25	46
1770 µg	(1500 nmol)	3.57 ± 0.17	165 ± 14	123 ± 28	42

Each value is a means ± SEM obtained from 6 individual animals. Sham animals were animals that underwent the surgical operation but not the co-arctation of the abdominal aorta. Control animals were animals that underwent coarctation of the abdominal aorta but were given saline instead of the peptide solution. [1]Hypertrophy Index = ventricle weight in mg/body weight in g. CBP = mean arterial blood pressure obtained from the carotid artery catheter, FBP = mean arterial blood pressure obtained from the femoral artery catheter, BP = CBP - FBP. *Significantly different from the control ($p < 0.05$, Student's t-test).

INDUSTRIAL APPLICABILITY

The industrial applicability of the invention is primarily in the medical or health care industry as an anti-cardiac hypertrophic agent in either the prevention or treatment of cardiac hypertrophy.

CLAIMS

1. Use of des-Aspartate-angiotensin I as an anti-cardiac hypertrophic agent in either the prevention or treatment of cardiac hypertrophy.

2. A pharmaceutical composition for preventing or treating cardiac hypertrophy, comprising:

an effective amount of des-Aspartate-angiotensin I; and

a pharmaceutically acceptable carrier or diluent.

3. A method for preventing or treating cardiac hypertrophy, which comprises:

administering to a subject in need of treatment an effective amount of des-Aspartate-angiotensin I.

4. A packaged pharmaceutical composition for preventing or treating cardiac hypertrophy, comprising:

a container suitable for storing a pharmaceutical preparation;

an effective amount of des-Aspartate-angiotensin I in said container; and

instructions associated with said container giving instructions for the use of said des-Aspartate-angiotensin I for preventing or treating cardiac hypertrophy.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG 96/00004

A. CLASSIFICATION OF SUBJECT MATTER

IPC[6]: A 61 K 38/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC[6]: A 61 K 38/08

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CAS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ENDOCRINOLOGY, Vol.108, No.2, February 1981 (Baltimore, USA), C. GARCIA DEL RIO et al.:"des-Asp-= Angiotensin I: Its Identification in Rat Blood and Confirmation as a Substrate for Converting Enzyme", pages 406-412; totality.	1
A	DE 39 26 606 A1 (HOECHST) 14 February 1991 (14.02.91), abstract.	1,2,4

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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Date of the actual completion of the international search	Date of mailing of the international search report
05 August 1996 (05.08.96)	23 August 1996 (23.08.96)

Name and mailing address of the ISA/ AT	Authorized officer
AUSTRIAN PATENT OFFICE Kohlmarkt 8-10 A-1014 Vienna	Wolf
Facsimile No. 1/53424/535	Telephone No. 1/53424/133

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG 96/00004

Box I **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 3
because they relate to subject matter not required to be searched by this Authority, namely:

Method for treatment the human body by therapy (see also Rule 39.1(iv) of the Regulations under the PCT).

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/SG 96/00004

In Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
DE A1 3926606	14-02-91	AT E 94409	15-10-93
		AU A1 60920/90	14-02-91
		AU B2 631914	10-12-92
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		ZA A 9006327	29-05-91

AN - 97-020931 [02]
AP - WO96SG00004 960522 EP960914540 960522; WO96SG00004 960522; [Based on WO9637213] WO96SG00004 960522; US970776026 970519; [Based on WO9637213]
PR - SG950000519 950525
TI - Use of des-aspartate-angiotensin I - as anti-cardiac hypertrophic agent in prevention or treatment of cardiac hypertrophy
- DES ASPARTATE ANGIOTENSIN ANTI CARDIAC HYPERTROPHY AGENT PREVENT TREAT CARDIAC HYPERTROPHY
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PN - WO9637213 A1 961128 DW9702 A61K38/08 Eng 013pp
- EP0774972 A1 970528 DW9726 A61K38/08 Eng 000pp
- US5773415 A 980630 DW9833 A61K38/00 000pp
IC - A61K38/00 ; A61K38/08
CT - 1.Jnl.Ref; DE3926606
AB - WO9637213 The use is claimed of des-aspartate-angiotensin I (Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu) (DAA) as an anti-cardiac hypertrophic agent in the prevention or treatment of cardiac hypertrophy.
- ADVANTAGE - DAA is a highly specific anti-cardiac hypertrophic agent and is effective at concns. that produce minimal or no secondary effects.

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